

Summary

This PhD Thesis deals with the systematic investigation of molecular dynamics and phase transitions in the case of hydrated proteins and other biomolecules, in a wide range of hydration levels. The main goal is the study of the dynamical characteristics of the observed transitions in relation to the coupling of hydration water to the biomolecules. In long terms, the main goal is to explain biological function in physical terms.

The hydrated systems studied in this thesis are: two globular proteins, bovine serum albumin (BSA) and lysozyme and two fibrous proteins, elastin and collagen. In addition to hydrated proteins, two structurally simpler biopolymers were chosen, in order to compare the results to the ones obtained for hydrated proteins as well as to literature results obtained on more simple synthetic polymers. In particular, these are, hydrated solid samples and aqueous solutions of casein peptone (a digest derivative of the protein casein), as well as hydrated solid hydrogels of hyaluronic acid (HA).

The crystallization and melting events of water and the glass transition were studied through differential scanning calorimetry (DSC). Molecular mobility (α relaxation of the hydrated systems and secondary relaxations of water in the uncrystallized and/or crystallized phase) was investigated in detail using two dielectric techniques, thermally stimulated depolarization currents (TSDC) and dielectric relaxation spectroscopy (DRS), in a wide frequency and temperature range. Additional information on the hydration properties of the samples was obtained by water equilibrium sorption measurements (ESI) at room temperature.

The thermal glass transition in the hydrated systems was recorded by DSC. Depending on the system the glass transition was recorded in different temperature regions, while it could not be detected for hydrated collagen. In general, the glass transition shows a strong hydration dependence. The variation of the glass transition temperature and the heat capacity step with hydration level leads to the conclusion that the motion associated with the transition includes contributions from the uncrystallized water molecules in the mixtures as well as segments of the biopolymer.

The dielectric response which is associated with the thermal glass transition was studied by DRS and TSDC. A relaxation which exhibits cooperative character, according to the temperature dependence of its dielectric strength, was recorded in quite good accordance with the results from calorimetry, in the case of less rigid systems. On the other hand, in the case of the fibrous protein elastin which exhibits a glass transition at higher temperatures than the rest of the systems, but also for the fibrous protein collagen, which does not exhibit a glass transition, a dielectric relaxation, similar to the dielectric manifestation of the glass transition previously mentioned for the rest of the systems, is still recorded. The striking result is that the relaxation in question is recorded in a specific temperature interval, independently of the calorimetric glass transition. It is suggested that the dielectric relaxation probes mainly the polarization of uncrystallized water molecules within clusters on the surface of the hydrated molecules.

The main dielectric relaxation due to uncrystallized water molecules in the hydration shell of the hydrated systems was systematically studied. An interrelation of the latter to a

Summary

secondary relaxation of polar groups on the surface of the hydrated systems was observed. In particular, the uncrystallized water molecules at low hydration levels trigger the motion of small polar groups on the surface of the biomolecules. The characteristic relaxation times are being reduced as the hydration level increases. The dynamical characteristics of the relaxation depend on the polar groups sequence and proportionality. In a critical region of hydration levels of about 20-30 wt% the surface of the biopolymer is gradually covered by water molecules and a percolative uncrystallized water cluster is formed, in parallel to the stabilization of both the relaxation time and the dielectric strength of the relaxation. In general, the relaxation is of secondary character and is well described by an Arrhenius law at low temperatures. In the region where the characteristics of the relaxation stabilize, the dielectric response corresponds to a mode within the uncrystallized water layer which covers the underlying surface. The excess water molecules beyond the main uncrystallized water layer, are being organized into various uncrystallized or crystalline forms depending on the structure. Finally, there are implications for a transition of the uncrystallized water molecules from a high density amorphous form (HDA) to a low density amorphous form (LDA) at high temperatures, at least in the case of the globular protein BSA.